IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF

MANLEY et al.

FILED: 5 January 2006

U.S. APPLICATION NO: 10/528,913

FOR: NOVEL PYRIMIDINEAMIDE DERIVATIVES AND THE USE THEREOF

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R 1.132

Sir:

I, Paul W. Manley, declare and state that:

1. A copy of my *curriculum vitae* demonstrating my education, training, and experience is attached. I am familiar with U.S. application Serial No. 10/528,913, and its prosecution history. I am considered by my peers to be an expert in the field to which the application pertains, and am otherwise qualified to speak and render expert opinions as to the present application, invention, and issues of the Office Action dated July 25, 2008. Thus, this declaration is in response to the Office Action.

2 It has been sought to demonstrate the inhibitory activity of a compound of the present invention with the compound and reference cited by the examiner, namely, example 11 ("prior art example") of WO 02/22597 to Buerger (Buerger '597). Example No. 17 of the present application differs from the prior art example in that there is a CF_3 group at position R3 of the pending claims where there is a hydrogen in the prior art example. In this manner, the potency of the presently claimed compounds as exemplified by example No. 17 of the present application, was compared with the potency of the prior art example.

3. The inhibitory properties of example 17 of the present application were compared in head-to-head comparison against M315I Imatinib-resistant mutant form of Bcr-Abl. The activity of the compounds against M315I is relevant because it demonstrates the ability of

the compound to inhibit imatinib-resistant mutant forms of Bcr-Abl. Thus, chronic myeloid leukaemia (CML) is caused by uncontrolled protein phosphorylation in hematopoietic stem cells that, as a result of a t(9,22) reciprocal chromosome rearrangement, carry the oncogenic BCR-ABL fusion gene that encodes the Bcr-Abl oncoprotein. Imatinib (Novartis Pharmaceuticals) is a drug that in cells inhibits the protein tyrosine kinase activity of Bcr-Abl and consequently is a highly effective therapy for patients diagnosed in the early chronic phase of this otherwise lethal cancer. However, patients with advanced stage disease frequently relapse due to the emergence of leukaemic cells expressing mutant forms of Bcr-Abl, having amino-acid substitutions within the kinase domain that render the enzyme less sensitive towards imatinib. One of the most common of these imatinib-resistant mutations is that in which the methionine 351 residue substituted by threonine (M351T) (Apperley, Lancet Oncol 2007;8:1018--29).

4. The following experiments were performed by me or under my direction, supervision, or control, and in the ordinary course of business.

5. The inhibition of phosphorylation of Bcr-Abl M351T can be determined by the same capture ELISA format as follows: The murine myeloid progenitor cell line Ba/F3 transfected to express either the p210 wild-type Bcr-Abl, or mutant forms such as M351T were obtained from J Griffin (Weisberg et al., Cancer Cell 2007;7:129-141). The cells express the fusion Bcr-Abl proteins having constitutively active abl tyrosine kinase domains and proliferate independently of growth factors. The cells are expanded in RPMI 1640 (AMIMED; cat # 1-41F01), 10% fetal calf serum, 2 mM glutamine (Gibco) ("complete medium"), and a working stock is prepared by freezing aliquots of 2 x 10^6 cells per vial in freezing medium (95% fetal calf serum, 5% dimethylsulfoxide (SIGMA, D-2650) After thawing, the cells are used during maximally 10 – 12 passages for the experiments. The antibody anti-abl SH3 domain cat # 06-466 from Upstate Biotechnology is used for the ELISA. For detection of Bcr-Abl phosphorylation, the anti-phosphotyrosine antibody Ab PY20, labelled with alkaline phosphatase (PY10(AP)) from ZYMED (cat. # 03-7722) is used. As comparison and reference compound, NVP-AMN107 (nilotinib, Novartis Pharmaceuticals), is used. A stock solution of 10 mM is prepared in DMSO and stored at –

20 °C. For the cellular assays, the stock solution is diluted in complete medium to yield a starting concentration of 20 or 6 µM followed by preparation of serial threefold dilutions in complete medium. No solubility problems are encountered using this procedure. The test compounds of formula I are treated analogously. For the assay, 200'000 Ba/F3 Bcr-Abl M351T cells in 50 µl are seeded per well in 96-well round bottom tissue culture plates. 50 µl per well of serial threefold dilutions of the test compound are added to the cells in triplicates. The final concentration of the test compound range e.g. from 10 µM down to 0.01 µM. Untreated cells are used as control. The compound is incubated together with the cells for 90 min at 37 °C, 5 % CO_2, followed by centrifugation of the tissue culture plates at 1300 rpm (Beckman GPR centrifuge) and removal of the supernatants by careful aspiration taking care not to remove any of the pelleted cells. The cell pellets are lysed by addition of 150 µl lysis buffer (50 mM Tris/HCl, pH 7.4, 150 mM sodium chloride, 5 mM EDTA, 1 mM EGTA, 1 % NP-40 (non-ionic detergent, Roche Diagnostics GmbH, Mannheim, Germany), 2 mM sodium ortho-vanadate, 1 mM phenylmethyl sulfonylfluoride, 50 µg/ml aprotinin and 80 µg/ml leupeptin) and either used immediately for the ELISA or stored frozen at –20 °C until usage. The anti-abl SH3 domain antibody is coated at 200 ng in 50 µl PBS per well to black ELISA plates (Packard HTRF-96 black plates; 6005207) overnight at 4 °C. After washing 3x with 200 µl/well PBS containing 0.05 % Tween 20 (PBST) and 0.5 % TopBlock (Juro, Cat. # TB 232010), residual protein binding sites are blocked with 200 µl/well PBST, 3 % TopBlock for 4 h at room temperature, followed by incubation with 50 µl lysates of untreated or test compound-treated cells (20 µg total protein per well) for 3-4 h at 4 °C. After 3 x washing, 50 µl/well PY20(AP) (Zymed) diluted to 0.5 µg/ml in blocking buffer is added and incubated overnight (4 °C). For all incubation steps, the plates are covered with plate sealers (Costar, cat. # 3095). Finally, the plates are washed another three times with washing buffer and once with deionized water before addition of 90 µl/well of the AP substrate CPDStar RTU with Emerald II. The plates now sealed with Packard Top Seal™-A plate sealers (cat. # 6005185) are in-

cubated for 45 min at room temperature in the dark and luminescence is quantified by measuring counts per second (CPS) with a Packard Top Count Microplate Scintillation Counter (Top Count). For the final optimized version of the ELISA, 50 µl of the lysates of the cells grown, treated and lysed in 96-well tissue culture plates, are transferred directly from these plates to the ELISA plates that are precoated with 50 ng/well of the rabbit polyclonal anti-abl-SH3 domain AB 06-466 from Upstate. The concentration of the anti-phosphotyrosine AB PY20 (AP) can be reduced to 0.2 µg/ml. Washing, blocking and incubation with the luminescent substrate are as above. The quantification is achieved as follows: The difference between the ELISA readout (CPS) obtained for with the lysates of the untreated cells and the readout for the assay background (all components, but without cell lysate) is calculated and taken as 100 % reflecting the constitutively phosphorylated Bcr-Abl protein present in these cells. The activity of the compound in the Bcr-Abl kinase activity is expressed as percent reduction of the Bcr-Abl phosphorylation. The values for the IC_{50} are determined from the dose response curves by graphical inter- or extrapolation.

The following table shows the mean IC_{50} values of example no. 17 of the present application compared to the prior art example

Compound	M351T BcrAbl mean IC_{50} ± SEM (n determinations)
Example 17 of present application	0.052 ± 0.022 n = 3
Prior Art Example	0.225 ± 0 067 n = 3

6. This data demonstrates that example no. 17 of the present application has superior potency against imatinib-resistant mutant form of Bcr-Abl, M315I, exhibiting a potency of greater than 2.5 times that of example 11 of Buerger '597. This is a feature of the trifluoromethyl group, which in addition making lipophilic interactions also participates in an electrostatic interaction with the Abl protein, which enhance the binding affinity of such molecules and contributes to their increased potency (Cowan-Jacob et al., Acta Cryst 2007;D63:80–93).

7. Therefore, it is respectfully submitted that the data contained herein directly refutes the rejection of claims 1-8 and 11 as being obvious over WO 92/22597 to Buerger et al. in the Office Action, and reconsideration and withdrawal of the rejection are solicited.

8. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further, that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Should the Examiner have any questions, please contact the undersigned attorney.

Date: 23 August 2009

Paul W. Manley